

Figure 1:

Amino acid sequences of Cpn60 and Cpn10:

SEQ ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):

MKIRPLHDRVVRRKEEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNIIADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTATVLAQAIISEGLKSVAAGMN
PMDLKRGIKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKM TVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLAILTGQGVISEELGMSLETADPSSLG TASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPK VTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGPOMM

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cacaaccgaagccatgggtgcggatgcgcctgtgaagaaggcgtgggtgtatgcctgatatgggcggcatgggtggaatgggcg
gtatgcctggcatgatgtaacacttltgtatcattgtcctgatctgcttaccgtgtaaaaagatcaggctcaaggctgtctctataaaaag
ccgtatctltgatgagtgttcttctgtctgaaaacgacattctggagtgcggctttttgtatttggtcataaaattcagaatatltgttaatt
ttatgtaactagctggcctataatgttgagttcccttgggtggcatgatctcatggtaactcacttaagcctgattcactgag
gctttaacagtaaaataataacgcaacgtagaaacataataagcgtatggcattaatgaagacggctgcatttaattcagatc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from *Oleispira antarctica* (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figure 4) 333 aa

MKNTLKSSSRFSLKQLGTGALHSSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLT YMERQGS DKN A ESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGG AISAIYSLSHPEKVKSL
TLIDAAGVDGDTESEYYKVL AEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAA AFKKIIPQATVHIFPEVGH LPMVEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of *Oleispira antarctica* (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene).

Positions 101-105 are *Bam*HI – *Sau*3A1 fusion and positions 3795-3798 are *Sau*3A1-*Bam*HI-fusion.

acaggaaacagctatgaccttgattacgccaagctcgaaattaaccctcactaaagggaaacaaaagctggagctcgcgccctgcag
gtcgacactagtggaalcaacggcggttcattggtactggctgaggtcagcgicataatgccgatggcgatactggccgctatgactgagtact
tcttctgtagcaaccgatttttctaatagcgcagcctcttatttctgaacgggcaactgatgtagtttttactaaccggccttttaggcaicgg
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gctctgattatctcagtttcttctcggtgggtgcaccacaacacaacagataatttatacacaggggttatgtctcttgcgagagacagc
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ttccacccgggtgggtaccaggtaaggtaccctaagtgagtgattatacaattcactggccgtcgttttac

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonins with the esterase gene (EstRB8), all from *Oleispira antarctica*

SEQ ID No 6: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 6) 97 aa:

MKIRPLHDRVVRKKEETATAGGHLPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYS GQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVNLA DAVRVT LGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQM VKEVASQANDQAGDGT TTTATVLAQAIISEGLKSVAAGMN
PMDLKR GIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANA DETVGR LIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLG TASKVVIDKENTVIVD GAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGV VAGGGVALIRALSSVT VVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGD MIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

SEQ ID No 8: estRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFSLKQLGTGALIISSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLT YMERQGS DKNDAESVILLHGFSADKDNWILFTKEFDEKYHVI AVDLAG
IIGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFH IAGNSMGGAISAIYSLSHPEKV KSL
TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAAFKKIIPQATVHIFPEVGHLP MVEIPSES AKVYEEFLSSIK

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with
esterase of *Oleispira antarctica* (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI*
site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and
positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector
(Stratagene)

Small letters -- the Cpn10-Cpn60 encoding fragment,

Capital italics -- fragments of vector pBK-CMV

Capital letters -- fragment coding for EstRB8 from plasmid pBK1Est

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCCTCACTAAAGGGA
ACAAAAGCTGGAGCTCtaataacttgggatccaacagttggagagictagcaaatgaaaatccgtccattacatgatcgtatt
gttcttcgccgtaaagaagaagagaccgcaactgcgggttggtatttaccgggcgtgcggcagaaaaaccaaatacaggtgtgt
tatctctgtgggtacttgcgttatttataatgggtcagtgcaagcgttgcgggttaacgaaggcgtgtgtctgttttggtaaatactc
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cggtaagaagggtgtgattaccgtgaagaaggcaaggccttgaagacgagcttgatgtgtgtagaaggcaatgcagctcgaatcgcgggt
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AATCGCAGTGGGTTTCTTGTTTTTCATCAACAGCAACAAACGTGAAATACCCCGTA
ATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACTTCAAC
TTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTTCGACAATGGTAC
CTGCGGGAACAGGATGCTTAAAATCGATTTCGATCACTGCTGACGGTTACGATGCT
TTGTCGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTGCATT
GCAGTGCCACCGAATAACGTATCATGATGATTGTGTCTCTGGAAATACCGCTTT
AGAAATAGTGGTTTTTGATACGCGCTTTTCGCTGCGCAATAATATCTTCTCTGCTAA
GAGTTGCGGATGGCATAACATAAACTCGCTTGATTAAAGATTAATAATAAATAGTTA
ACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACTTTGAG
GCCGCTAGAGAGAAAAAGACCAGTGATAATATTTCATCTTGCCATGAGAGCTTATC
ATGAAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGAAATAA
TACCAATATATTTTCATATATAATTTACACTACCCCTTATCTCACTAGACTTCCCGC
GCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTTCGCTGCAACAC
ATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCCTT
TGCGATTTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTCTAACC
ACCACCATTGGTTCTTTTCCACAAACTCAAAAAACTCGTCAAATCCGCTTGCAATT
TAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATT
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ACAATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTTAAT
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TTCCAGCCAATGGGACTGATTTCATGGCGGCTCAAATGTATTGCTGGCAGAAACAC
TGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTTGTGTTGG
CCAAGAAATTAACGCCAACCACATACGCGGTGTTTCGTTCCGGCATAGTGACTGGC
ACAGCAACGCTAGTACACAAAGGAAGAACCCTCCAGATTGCGGAAATTCGCATC
GTTAACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGG
CCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGT
GAGTCGATTACAATTCCTGCGCGTCGTTTTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

SEQ ID No 10: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 8) 97 aa:

MKIRPLHDRVVRRKEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

Below – **Capital bold letters** are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVN^{IL}ADAVR^{VT}LGPKGRNVVIEKSFGAPIITKDGVS^V
AREIELKDKFENMGAQM^VKEVASQANDQAGDGT^{TT}TATVLAQAIISEGLKSVAAGMN
PMDLKR^GIDKATAAVVAAI^{KE}QAQPCLDTKAIAQVGTISANADETVGR^LIAEAMEKV
GKEGVITVEEGKGLEDEL^DVVEGMQFDRGYLSPYFINNQE^KMTVEMENPLILLVD^{KK}
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA^{AV}KAPGFGD
RRKAMLQDLA^{IL}TGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVD^GGAGTEAS
VNTRVDQIRAEIES^{TS}DYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGV^VVAGGGVALIRALSSVT^VVGDNEDQNVGIALALRAMEAPIRQI
AGNAGA^{AG}44VVDKVKSGTGSFGFNASTGEYGD^{MI}AMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGM^{PG}MM

SEQ ID No 12: EstRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 8) 333 aa:

MKNTLKSSSRFSLKQLGTGAL^{ISS}LF^{FG}GCTTTQQDNLYTGVM^{SL}ARDSAGLEVKTA
SAGDVNLTYMERQGS^{DK}DNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT^{TD}YGLIKQAERLDIFLSGLGVNSFH^{IA}GNSMGG^{AI}SAIYSLSHPEKVKSL
TLIDAAGVDGDTESEYYK^VLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL

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RKTLARAEINNKFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAAFKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *Sall* site (pos. 2138-2143).

In the DNA sequence:

Small letters --- the Cpn10-Cpn60 coding fragment,

Capital italics -- fragments of vector

Capital letters -- fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCTCACTAAAGGGA
ACAAAAGCTGGAGCTCetaatactgggatccaacaggttgagagctagcaaatgaaaatecgtecatcatgatcgtatt
gttgtegcgtaaagaagaagagaccgcaactgcgggtggtattatitacgggcgctgcggcagaaaaacaaatcaaggigtgt
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ATTGAAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTC
AAATTACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACG
TACCTTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAA
ACACTGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTG
TTGGCCAAGAAATTAACGCCAACCACATACGCGGTGTTTCGTTCCGGCATAGTGAC
TGGCACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTCCG
CATCGTTAACGATCCAAAGAATTCAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCG

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CGGGCCCATCGATTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCT
ATAGTGAGTCGTATTACAATTCACCTGGCCGTCGTTTTAC

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751 of Figure 10):

MKIRPLHDRVVRRKEEETATAGGHLPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYS GQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVN ILADAVRVT LGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGT TATVLAQAIISEGLKSVAAGMN
PMDLKR GIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANA DETVGR LIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGQQVISEELGMSLETADPSSLG TASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVT VVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

Figure 10:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant
Glu460Ala/Ser462Ala/Val463Ala:

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Big bold letters = introduced mutations

atcaaaaaatgcagcaaggacagattccgcccagaafagcagaaggffcttctgttagcaatggccggcgcttattataacgccgg
gtttgtcactgatgcgctgggtttacattactcgccccgcgacgcgtaaagcgttggccataagggtattgcatttattaccctc
gcatgatgactgcaagcagcttcaagcgacgggtagtttcaggaaggctcgttfaaagatgtacatcgcacacgactcgcaagca
gtcatgaaaaaatcacaattgaaggcgaatataccaaagacgataagtaggtatttttggctagccgtgaaacctagtaaaagccc
cgafaaaitaaccatctattttcacagaggcaatttagcccttgtttaccitattgafectaatacttgggatccaacagttggagagcttagc
aaatgaaaatccgtccattacatgatcgtattgttgcgcgfaaagaagaagagaccgcaactgcgggtgggtattatttacc
ggcgctgcggcagaaaaacaaatcaagggtgtgtatctctgtgggtactggccgtattcttgataatggttcagtgcgaagcgtggc
ggtaacgaaggcgatgttgcgttttgglaatactcaggtcaaaatactatcgataatcgatgggtgaagaattaitgatttgaatga
aagtgtatctacggcggtttagaagcttaattattacactcacctttttttaacctacaaaatttaaggaaagatcatggctgttaaagacg
tattatttgggtgatagcgacgcgcaaaaatgttggtaggtgtaaacatttagccgacgcagtaagagttaccttaggacctaa
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gtactagcacaggcgattatcagcgaagggttgaatctgttgcggctggcatgaatccaatggatctfaaactgttgaataagcta
cggctgtctgttgcgccaitaagaacaagctcagcctgttggatacaaaagcaatcgctcaggtagggacaactctctgccaatg
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gatcacaaccgaagccatgggtgcggatgcgectgtgaagaaggcgctgggtgatgcctgatatggcgggcatgggtggaatggg
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gctttaacaglaaaataaataacgcaacgtagaaaataaataagcgtatggcatfaatgaagacggcctgcatttaattcagatc